THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Polymicrogyria

Clinical Features:

Polymicrogyria (PMG) is a cortical brain malformation which is characterized by an excessive number of small irregular gyri separated by shallow sulci, which leads to an irregular cortical surface [1]. PMG varies widely in extent and location in the brain depending on the underlying etiology or syndrome, and can be isolated to a single region of one hemisphere, bilateral and asymmetric, bilateral and symmetric, or diffuse [1]. Depending on the extent, subtype, and underlying etiology of PMG, clinical manifestations may range from selective impairment of cognitive function to severe encephalopathy with intractable epilepsy [1]. PMG may be isolated, or observed as part of a multiple congenital anomaly syndrome. It may be associated with a genetic etiology, or may be due to exogenic causes such as infection, or impaired hemodynamic disturbances [1].

Our Polymicrogyria Panel includes mutation analysis of the 24 genes listed below.

Polymicrogyria Panel Genes				
AKT3	GPR56	OCLN	RTTN	TUBB
BICD2	GPSM2	PIK3R2	SNAP29	TUBB2B
CCND2	KIAA1279	RAB18	TBC1D20	TUBB3
COL18A1	NDE1	RAB3GAP1	TUBA1A	WDR62
FIG4	NEDD4L	RAB3GAP2	TUBA8	

Gene / Condition	Clinical and Molecular Findings
AKT3 [OMIM # 611223] Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 2 [OMIM 615937]	Heterozygous *de novo* mutations in the *AKT3* gene have been associated with Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 2 [2,3]. Somatic mosaicism for mutations in the *AKT3* gene have been associated with hemimegalencephaly; these variants may not be detected unless pathological tissue is tested and the mutation is present in a high percentage of cells in that tissue [2].
BICD2 [OMIM# 609797] Arthrogryposis multiplex congenital and polymicrogyria	A recurrent *de novo* variant in *BICD2* has been reported in two unrelated patients with arthrogryposis multiplex congenital, hypotonia and bilateral perisylvian polymicrogyria [4]. Other variable features included congenital fractures, hip dislocation, micrognathia, respiratory insufficiency, and microcephaly.
CCND2 [OMIM# 123833] Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome [OMIM# 615938]	Heterozygous mutations in *CCND2* have been reported in patients with megalencephaly, polymicrogyria, polydactyly and hydrocephalus (MPPH) [5]. One of the affected individuals had a mother with large head circumference and borderline intelligence, who was found to carry a *CCND2* variant in the mosaic state.
COL18A1 [OMIM# 120328] Knobloch syndrome [OMIM# 267750]	Biallelic mutations in *COL18A1* have been associated with Knobloch syndrome, which is typically characterized by eye abnormalities such as vitreoretinal degeneration and retinal detachment and occipital skull defects such as occipital encephalocele. More recently, multiple unrelated cases of biallelic *COL18A1* mutations associated with polymicrogyria, in addition to other findings such as epilepsy, myopia and retinal detachment, have been reported [6-8].
FIG4 [OMIM# 609390] Bilateral temporooccipital polymicrogyria [OMIM# 612691]	A homozygous mutation in the *FIG4* gene has been reported in one consanguineous family with bilateral occipital polymicrogyria. Studies of FIG4-null mice showed post-migration brain abnormalities, consistent with the mechanism underlying polymicrogyria in humans [9].

GPR56 [OMIM# 604110] Bilateral Frontoparietal Polymicrogyria [OMIM# 606854]	Bilateral frontoparietal polymicrogyria (BFPP) consists of polymicrogyria with multiple and fused small gyri, an irregular limit between white and grey matter, white matter abnormalities and cerebellar hypoplasia [10]. These radiological findings overlap with the features observed in cobblestone complex brain malformations such as muscle-eye-brain disease [OMIM#613153] [10]. *GPR56* encodes a G protein-coupled receptor which is thought to be involved in regulating the maintenance of the pial basement membrane integrity in the forebrain and cerebellum [10]. Bahi-Buisoon *et al*. (2010) identified *GPR56* homozygous mutations in 15 out of 30 patients with radiological findings of BFPP. *GPR56* mutations are associated with clinical findings of hypotonia and pseudomyopathic behavior, moderate to severe intellectual disability, seizures, abnormal eye movements and bilateral pyramidal and cerebellar signs [10].
GPSM2 [OMIM# 609245] Chudley-McCullough syndrome [OMIM# 604213]	Chudley-McCullough syndrome (CMS) is an autosomal recessive condition that is characterized by early onset severe to profound sensorineural hearing loss and brain abnormalities including frontal polymicrogyria, partial agenesis of the corpus callosum, grey matter heterotopia, and cerebellar dysplasia [11]. Cognitive impairment and seizures are rarely reported in individuals with CMS. Patients with CMS have been found to have compound heterozygous or homozygous mutations in the *GPSM2* gene, including frameshift, nonsense, and splice-site mutations [11]. The GPSM2 protein is involved in regulating the orientation of the mitotic spindle during cell division [12].
KIAA1279 [OMIM# 609367] Goldberg-Shprintzen Megacolon syndrome [OMIM# 609460]	Goldberg-Shprintzen syndrome (GOSHS) is an autosomal recessive multiple malformation disorder characterized by Hirschsprung megacolon, microcephaly, hypertelorism, submucous cleft palate, short stature, and intellectual disability [13]. Brooks *et al.* (2005) identified a homozygous nonsense mutation in *KIAA1279* in all affected individuals of a Moroccan family with polymicrogyria and a clinical diagnosis of GOSHS. The function of the KIAA1279 protein product is unknown, however its mRNA has been identified as localizing in the adult central nervous system, including in the cerebellum [13].
NDE1 [OMIM#609449]	Mutations in *NDE1* have been reported in children with severe congenital microcephaly, with brains smaller than 10 SD below the mean, with simplified gyri, and profound developmental handicap with normal body growth. Patients may also have lissencephaly or microhydraencephaly. Paciorkowski, *et al.* (2013) reported a patient with a full gene deletion and a truncating mutation in *NDE1* who had severe microcephaly, agenesis of the corpus callosum, and a cortical dysplasia with a polymicrogyria-like appearance [14]. NDE1 is highly expressed in the developing human and mouse cerebral cortex, particularly at the centrosome, and has a role in mitotic spindle assembly during early neurogenesis. Deficiency of NDE1 therefore appears to cause failure of neurogenesis and a deficiency of cortical lamination [15].
NEDD4L [OMIM# 606384] Periventricular nodular heterotopia 7 [OMIM# 617201]	*De novo* missense mutations in *NEDD4L* have been reported in multiple patients with periventricular nodular heterotopia, a form of cortical malformation[16]. One patient with a *de novo NEDD4L* mutation and polymicrogyria in addition to periventricular nodular heterotopia has been reported [17].
OCLN [OMIM# 602876] Band-Like Calcification with Simplified Gyration and Polymicrogyria [OMIM#251290]	Band-like calcification with simplified gyration and polymicrogyria (BLC-PMG) is a rare autosomal recessive disorder characterized by bilateral, symmetrical polymicrogyria, a prominent band of gray matter calcification on brain imaging, and calcification in the cerebellum and basal ganglia [18]. Clinical features include early onset seizures, severe microcephaly and developmental arrest. O'Driscoll *et al.* (2010) identified *OCLN* mutations in 9 patients from 6 families with a BLC-PMG phenotype. *OCLN* encodes for occludin, which is a key component of tight junctions in the brain, which are functional in cerebral blood vessels in early fetal development [18].
PIK3R2 [OMIM# 603157] Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome [OMIM# 603387]	Heterozygous and mosaic variants in *PIK3R2* have been described in patients with phenotypes ranging from bilateral perisylvian polymicrogyria to megalencephaly-polymicrogyria-polydactyly-hydrocephalus (MPPH) syndrome [19]. *Our sequencing assay is designed to detect heterozygous variants, variants present in the mosaic state may not be detected by this assay.*
RAB18 [OMIM#602207] *RAB3GAP1* [OMIM#602536] *RAB3GAP2* [OMIM#609275]	Warburg Micro syndrome [OMIM #600118] is a rare autosomal recessive condition characterized by ocular and neurodevelopmental abnormalities and hypothalamic hypogonadism [20,21]. Key clinical features include microphthalmia, microcornia, congenital cataracts, optic atrophy, microcephaly, cortical dysplasia and atrophy, congenital hypotonia, severe intellectual disability, and spastic diplegia [20,21]. Progressive joint contractures, growth failure, kyphoscoliosis and hypertrichosis have also been

TBC1D20 [OMIM# 611663] Warburg Micro syndrome [OMIM#600118]	described in a proportion of affected individuals [20]. In addition to the characteristic ocular findings, common facial features include deep set eyes, wide nasal bridge and a narrow mouth [20]. Brain magnetic resonance imaging (MRI) of affected individuals consistently shows polymicrogyria in the frontal and parietal lobes, wide sylvian fissures, thin corpus callosum and increased subdural spaces [20].
RTTN [OMIM#610436] Polymicrogyria with seizures [OMIM#614833]	Kheradmand Kia et al (2012) identified a homozygous mutation in RTTN in three members of a consanguineous family with polymicrogyria and seizures [22]. The polymicrogyria in these affected individuals was asymmetric extending from the frontal to the temporal, parietal and occipital lobes on brain MRI. RTTN is required for the early development of left-right specification and axial rotation and may play a role in notochord development.
SNAP29 [OMIM# 604202] Cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma syndrome [OMIM# 609528]	Biallelic mutations in *SNAP29* have been described in patients with CEDNIK (cerebral dysgenesis, neuropathy, ichthyosis, and keratoderma) syndrome. Affected individuals have abnormalities of the corpus callosum and varying degrees of cortical dysplasia including polymicrogyria and pachygyria [23,24].
TUBA1A [OMIM#602529]	Poirier et al, identified mutations in *TUBA1A* in 3/95 sporadic patients with non-syndromic bilateral PMG. These patients had bilateral perisylvian asymmetrical PMG with dysmorphic basal ganglia cerebellar vermian dysplasia and pontine hypoplasia [25].
TUBA8 [OMIM# 605742] Polymicrogyria with Optic Nerve Hypoplasia [OMIM#613180]	Abdollahi et al. identified homozygous *TUBA8* mutations in two consanguineous families with extensive bilateral polymicrogyria and optic nerve hypoplasia. Clinical findings in the affected individuals included severe developmental delay, hypotonia and seizures [26]. The affected individuals did not have any noted dysmorphic features [26]. The TUBA8 protein is widely expressed in neural tissues, and is thought to have a role in cortical organization and regulation of brain development [26].
TUBB [OMIM# 191130] Cortical dysplasia, complex, with other brain malformations 6 [OMIM# 615771]	The *TUBB gene* is highly expressed in the developing cortex and heterozygous mutations in this gene cause microcephaly in addition to a range of structural brain abnormalities that include simplified gyral pattern to focal polymicrogyria [27].
TUBB2B [OMIM #612850] Asymmetric Polymicrogyria [OMIM #610031]	Patients with TUBB2B mutations typically have bilateral, asymmetric polymicrogyria, which is more striking the frontal and temporal lobes [28]. Other findings on MRI include absence of the corpus callosum, abnormal basal ganglia and cerebellum, and hypoplasia of the brainstem [28]. Most patients also have microcephaly, severe mental retardation and seizures [28]. Mutations of the TUBB2B gene, or α-tubulin, have been identified in patients with asymmetrical polymicrogyria [28]. TUBB2B is expressed in post-mitotic neurons during neuronal migration and differentiation [29]. Jaglin et al. (2009) reported four unrelated individuals and one fetus with asymmetrical PMG and autosomal dominant de novo mutations in TUBB2B [28].
TUBB3 [OMIM #602661] Complex Cortical Dysplasia with Other Brain Malformations [OMIM#614039]	Complex cortical dysplasia with other brain malformations (CDCBM) is a neuronal migration disorder associated with axon guidance defects. Clinically, patients have mild to severe intellectual disability, strabismus, axial hypotonia, and spasticity [30]. Cortical malformations seen on brain MRI include polymicrogyria, gyral disorganization, fusion of the basal ganglia, thin corpus callosum, hypoplastic brainstem, and abnormal cerebellar vermis [30]. Autosomal dominant mutations of the TUBB3 gene were reported in 10% (12/120) of patients with CDCBM who were previously negative for mutations in LIS1, DCX, TUBA1A, TUBB2B, and GPR56. TUBB3 encodes a neuronal betatubulin subunit [30].
WDR62 [OMIM#613583]	Mutations in WDR62 have been reported in a subset of patients with microcephaly, cortical malformations, and moderate to severe ID. Besides microcephaly, these patients had various brain malformations including callosal abnormalities, polymicrogyria, schizencephaly and subcortical nodular heterotopia. A subset has seizures [31]. Homozygous missense and frameshift mutations were first reported in seven consanguineous families. Like other autosomal recessive primary microcephaly genes, WDR62 encodes a spindle pole protein that is expressed in neuronal precursor cells undergoing mitosis in the proliferative phase of neurogenesis [32].

Inheritance:

COL18A1, FIG4, GPR56, GPSM2, KIAA1279, NDE1, OCLN, RTTN, *TBC1D20, TUB8A, RAB18, RAB3GAP1, RAB3GAP2, SNAP29, TBC1D20,* and *WDR62* mutations are inherited in an autosomal recessive pattern. Parents of an affected child are most likely obligate carriers. Recurrence risk for carrier parents is 25%.

AKT3, BICD2, CCND2, NEDD4L, PIK3R2, TUBA1A, TUBB, TUBB2B and *TUBB3* mutations are inherited in an autosomal dominant pattern. All *TUBB2B* mutations described to date have been *de novo* in nature. The recurrence risk for parents is less than 1%, based on the theoretical risk for germline mosaicism. Both *de novo* and inherited mutations in *TUBB3* have been described. The recurrence risk for unaffected parents of an isolated case is <1%. The recurrence risk for affected parents is 50%. Mosaicism has been reported in the parent of a child with a *CCND2* mutation. Mosaicism for variants in the *AKT3* gene has been associated with hemihypertrophy phenotypes.

Test methods:

Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Sequencing may not detect low level mosaicism.

Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Polymicrogyria Panel (24 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2500
CPT codes:	81406 81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for the above test.

Results:

Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

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